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Mediator1: An Important Intermediary of Vitamin D Receptor–Regulated Epidermal Function and Hair Follicle Biology

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Considerable data in the literature support the idea that 1,25-dihydroxyvitamin D_3 and the vitamin D receptor (VDR) are involved in regulating skin biology. Studies using cultured keratinocytes, artificial human skin, and transgenic mouse models, as well as observations in patients with rickets, provide evidence of this pathway's importance in epidermal proliferation and differentiation and the hair growth cycle. The report by Oda *et al.* in this issue also indicates an important role of the VDR coactivator mediator 1 in these processes.

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Vitamin D can be obtained from the diet or produced in the skin from 7-dehydrocholesterol as the result of exposure to sunlight (UVR). This sterol must then be hydroxylated twice, in sequence at the 25 and 1 positions, to form the active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). For the production of systemic $1,25(OH)_2D_3$, the hydroxy group at the 25 position is added mainly in the liver, whereas the rate-limiting 1α-hydroxylation reaction occurs in the kidney under control of the parathyroid hormone. The role of $1,25(OH)_2D_3$ in the regulation of mineral homeostasis is well known. In addition to being the site for UV-induced generation of vitamin D, epidermal keratinocytes express both of the enzymes—vitamin D 25-hydroxylase (*CYP2R1*) and 25-hydroxyvitamin D 1α-hydroxylase (*CYP27B1*)—required for the production of active $1,25(OH)_2D_3$. Thus, keratinocytes have the complete machinery to produce the active metabolite of vitamin D upon exposure of skin to UVR (reviewed in Bikle, 2010). Moreover, these cells express the receptor for $1,25(OH)_2D_3$, the vitamin D receptor (VDR). VDR is a nuclear hormone receptor that heterodimerizes with the retinoid X receptor (RXR) to regulate transcription of genes that possess vitamin D response elements in their promoters (reviewed in Bollag, 2007). Several additional proteins are involved in VDR's regulation of transcription, including coactivators such as mediator1 (MED1) and steroid receptor coregulator 2 (SRC2) and 3 (SRC3).

The importance of the vitamin D pathway in epidermal keratinocytes has been shown in mouse and human keratinocytes in culture (reviewed in Bikle, 2010). Such studies have shown that $1,25(OH)_2D_3$, or its analogs, can inhibit keratinocyte proliferation and promote differentiation. Furthermore, decreasing the levels of members of the pathway (e.g., VDR or its coactivators) reduces keratinocyte differentiation *in vitro* (Amor *et al.*, 2010). These results have been extended into artificial human epidermis (Hawker *et al.*, 2007), in that knockdown of VDR, MED1 (referred to as vitamin D receptor–interacting protein 205 or DRIP205 here), SRC2, or SRC3 using adenovirus-delivered short hairpin RNAs resulted in reductions in various keratinocyte differentiation markers following generation of an epidermal-like structure in an advanced human keratinocyte differentiating system (reviewed in Bollag, 2007). The presumed transcription factor hairless interacts with the VDR and suppresses VDR-mediated transcription, and knockdown of hairless increases VDR-induced keratinocyte differentiation (reviewed in Bikle, 2010).

Additional studies have shown the importance of the vitamin D pathway in regulating skin biology *in vivo* using transgenic mouse models. For example, *VDR*-null mutant mice exhibit reduced keratinocyte differentiation marker levels in the interfollicular epidermis and hair loss that begins with the second hair cycle after birth (i.e., during postnatal maintenance of the hair cycle rather than being initiated during the first cycle, which represents completion of embryologic development of the hair follicle) (Xie *et al.*, 2002). This result may be attributable to the key role of the VDR in keratinocyte stem cell function (Cianferotti *et al.*, 2007). Alopecia in the *VDR* knockout mice could not be prevented by a high-calcium diet that blocks the bone and mineral homeostasis defects observed in these mice (Li *et al.*, 1998). However, hair loss in the *VDR*-null mice was prevented by expression of keratinocyte-targeted VDR (Chen *et al.*, 2001), even one with a mutation in the ligand-binding domain (Skorija *et al.*, 2005). This finding suggests that the effects of VDR in regulating hair follicle biology are ligand-independent. On the other hand, restoration of VDR expression in keratinocytes using a construct with a mutation in the DNA binding domain did not prevent the alopecia that results from global deletion of the *VDR* gene (Skorija *et al.*, 2005).

The ligand independence of VDR with respect to its effects on hair follicle maintenance is further supported by the phenotype of transgenic mice lacking the gene encoding 25-hydroxyvitamin D 1α-hydroxylase (*CYP27B1*), the enzyme

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Table 1. Summary of the effects of the loss of vitamin D pathway genes on epidermal biology

	Proliferation in IFE	Differentiation markers in IFE	Hair loss
MED1 KO (Oda *et al*., 2012)	↑	↑	Yes
CYP27B1 KO (Bikle *et al*., 2004)	No change	↓	No
VDR KO (Xie *et al*., 2002)	No change	↓	Yes
VDR KO + high-calcium rescue diet (Li *et al*., 1998)	NR	NR	Yes
VDR KO + KC-VDR (Chen *et al*., 2001)	NR	NR	No
VDR KO + KC-VDR with mutant ligand-binding domain (Skorija *et al*., 2005)	NR	NR	No
VDR KO + KC-VDR with mutant DNA binding domain (Skorija *et al*., 2005)	NR	NR	Yes
Hairless KO (Zarach *et al*., 2004)	↑	↑	Yes
RXR conditional KO (Li *et al*., 2001)	↑	↓	Yes

IFE, interfollicular epidermis; KC-VDR, keratinocyte-targeted VDR; KO, knockout; NR, not reported; VDR, vitamin D receptor.

that generates active $1,25(OH)_2D_3$. These mice do not develop alopecia, although they do exhibit reduced levels of keratinocyte differentiation markers in the interfollicular epidermis, as well as impaired permeability barrier homeostasis (Bikle *et al*., 2004). By contrast, spontaneous mutations in the VDR suppressor *hairless* (see above) in mice result in—as the gene name implies—hair loss (Xie *et al*., 2006). A *hairless* knockout mouse model also exhibits hair loss, as well as increased proliferation and enhanced expression of markers of keratinocyte differentiation (Zarach *et al*., 2004). Ablation of the VDR heterodimer partner RXR in *RXR*-null mutant mice also largely reproduces the hair-loss phenotype of the *VDR* knockout mice (Li *et al*., 2001).

There is also evidence in humans of the importance of vitamin D and VDR in skin biology. $1,25(OH)_2D_3$ and its analogs have been used successfully to treat psoriasis (Amor *et al*., 2010), a skin disorder characterized by keratinocyte hyperproliferation and abnormal differentiation, inflammation, and vascular changes. As indicated earlier, the role of $1,25(OH)_2D_3$ in the regulation of mineral homeostasis and bone physiology is well known, and vitamin D deficiency results in rickets (i.e., weak bones). Similarly, mutations in *VDR* lead to an inherited form of rickets, type IIA vitamin D–dependent rickets. Patients with this form of rickets also exhibit alopecia, suggesting the importance of the vitamin D pathway in regulating hair follicle biology in humans (Amor *et al*., 2010).

In a study reported in this issue, Oda *et al*. generated a conditional knockout mouse model lacking the *MED1* gene in the epidermis. MED1 is a coactivator known to interact with the VDR, and previous studies have shown its importance in responses initiated by VDR as well as in keratinocyte differentiation (Hawker *et al*., 2007). Oda *et al*. found that ablation of MED1 results in hair loss and increased proliferation, as well as elevated expression of differentiation markers in interfollicular epidermis. Although similar to the phenotype observed with the *hairless* knockout mouse model (Zarach *et al*., 2004), this latter finding was perhaps unexpected, but it points to possible unique actions of MED1 relative to its partner molecules within the vitamin D pathway. Again, the first developmental hair cycle was not affected, but later postnatal maintenance cycles were, suggesting the possibility that defects in MED1 function may also contribute to alopecia in certain patient populations. The results from these various mouse models are summarized in Table 1.

There is an additional phenotypic characteristic worth mentioning that has been observed in the *VDR* knockout mouse model. These mice show enhanced sensitivity to both chemical carcinogenesis (Zinser *et al*., 2002) and UVR-induced tumor formation (Ellison *et al*., 2008). Interestingly, *CYP27B1* knockout mice do not exhibit increased susceptibility to UV-elicited tumorigenesis (Ellison *et al*., 2008).Thus, in addition to playing key roles in epidermal and hair biology, VDR also seems to exhibit a tumor suppressor function, apparently independent of binding of the $1,25(OH)_2D_3$ ligand. Clearly, additional studies are needed to fully define the role(s) of the members of the vitamin D pathway in regulating various aspects of skin physiology, as well as the involvement of these proteins in skin diseases, including alopecia. The report by Oda *et al*. represents a promising foray in this direction.

CONFLICT OF INTEREST

The author states no conflict of interest.

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Clinical Implications

- There is evidence in humans of the importance of vitamin D and vitamin D receptor in skin biology.
- Keratinocytes have the complete machinery to produce the active metabolite of vitamin D upon exposure of skin to UVR.
- Patients with vitamin D–dependent rickets IIA may exhibit alopecia, suggesting a role for the vitamin D pathway in regulating hair follicle biology in humans.

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Homeward Bound: How Do Skin Dendritic Cells Find Their Way into the Lymph System?

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The success of the adaptive immune system relies upon the transport of antigens by dendritic cells (DCs) from skin via lymphatic vessels to lymph nodes where DCs present antigen to T cells. Little is known about the requirements for so-called "reverse transmigration" (RT). Torzicky *et al.* in this issue demonstrate that CD31, CD99, and CXCR4 play key roles in RT, suggesting that adhesion occurs in a defined sequence during the passage of DC into lymphatics.

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The success of the adaptive immune system relies in part on the efficient transport of antigens by specialized antigen-presenting cells (e.g., dendritic cells (DCs) from barrier tissue such as skin (where infectious organisms first invade) to secondary lymphoid organs such as lymph nodes (LNs), where the DCs present antigen to naive and memory T cells (Randolph *et al.*, 2005). Although this process is critical for immune cell priming, much less is known about it, including the cytokines and adhesion molecules that are required, than about the effector phase of adaptive immunity in which T cells (and other leukocytes) migrate from the blood stream to tissue by first transiently stopping (rolling), firmly arresting, and finally crawling (diapedesis) between blood vascular endothelial cells (BVECs) to emerge on the tissue side of the blood vessel (Mackay, 1999).

Lymphatic vessels have long been appreciated in humans. Veiled cells resembling macrophages were identified more than 40 years ago in lymphatic fluid (Smith *et al.*, 1970). These veiled cells were eventually recognized to be potent antigen-presenting cells called DCs; in skin, they are composed primarily of epidermal DCs called Langerhans cells (LCs) and dermal DCs.

The complex route of epidermal LCs has been of particular interest because these cells must leave the epidermis prior to entering lymphatic vessels. Migration of LCs appears to be associated with the activation of resting LCs by inflammatory triggers such as IL-1β and tumor necrosis factor-α. The latter two cytokines not only cause LCs to strongly upregulate major histocompatibility complex class II and costimulatory molecules but also may induce the generation of lymphangiogenic cytokines such as vascular endothelial growth factor-C, which increase the number of lymphatic vessels at sites of inflammation. As LCs mature, they also downregulate expression of E-cadherin (Schwarzenberger and Udey, 1996), which may anchor LCs to keratinocytes in the epidermis and initiate expression of matrix metalloproteinases, allowing them to pass through the basement membrane (Angeli and Randolph, 2006).

Two key membrane receptors that are upregulated with LC/DC maturation are the chemotactic receptors CC chemokine receptor-7 (CCR7) and CXC chemokine receptor-4 (CXCR4). The role of CCR7 and its two ligands (CCL19 and -21) in lymphatic trafficking is better understood because CCR7-deficient knockout mice (CCR7-KO) (Foerster *et al.*, 1999) and paucity of LN T cells (*plt*) mice (Gunn *et al.*, 1999), which lack expression of select CCR7 chemokine ligands (CCL19/21) at the periphery and within LNs, both exhibit small peripheral LNs. This phenotype

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